Personal Reflections on 50 Years of Study of Benzene Toxicology

Dennis V. Parke

School of Biological Sciences, University of Surrey, Guildford, Surrey, England

The metabolism of benzene is reviewed, and the objectives of a quantitative balance study begun in 1945 are outlined; problems of toxicology and metabolism research of some 50 years ago are considered. The quantitative metabolism of ^{14}C-benzene in the rabbit is annotated and compared with that of unlabeled benzene quantified by nonisotopic methods. The anomalies of phenylmercapturic acid and *trans-trans*-muconic acid as metabolites of benzene are examined in detail by isotopic and nonisotopic methods; these compounds are true but minor metabolites of benzene. Oxygen radicals are involved in both the metabolism of benzene and its toxicity; the roles of CYP2E1, the redox cycling of quinone metabolites, glutathione oxidation, and oxidative stress in the unique radiomimetic, hematopoietic toxicity of benzene are discussed. Differences between the toxicity of benzene and the halobenzenes are related to fundamental differences in their electronic structures and to the consequent pathways of metabolic activation and detoxication. — Environ Health Perspect 104(Suppl 6):1123–1128 (1996)

Key words: benzene, myelotoxicity, oxygen radicals, CYP2E1, metabolism, historical perspectives

Introduction

My first insight into benzene toxicity came in the autumn of 1939 when, as a young first-year medical student at University College, London, I was sent to work on a research project at Glaxo Laboratories, Greenford, U.K. Glaxo had been given the wartime task of manufacturing penicillin and a host of other drugs and vitamins needed for the war effort; in return they had been given the services of numerous science graduates who otherwise would have been conscripted into the armed services. In those days of World War II, full-time university students were exempt from conscription but were encouraged to undertake part-time war work in addition to their studies; hence my casual employment at Glaxo, assisting the works' medical officer to ascertain why so many of the research workers were showing clinical signs of scurvy. Ascorbate excretion and loading studies confirmed the vitamin C deficiency; and it quickly became apparent that this was associated with high exposure to benzene, which was the major solvent used in the various production lines. Open vats containing 100 litres or more of benzene solutions were moved around on trolleys. Often the solvent was spilled, so the concrete floors were awash with benzene and the workers' clothes were soaked in the solvent. In addition, benzene was continuously being distilled in open systems to recover the solvent for further use, and serious conflagrations were all too frequent. Strangely, although it was well documented at that time that exposure to benzene was associated with aplastic anemia, none of the chemists employed considered that there was any health hazard in their negligent use of this solvent.

When the exposure problems were brought out, the Glaxo management acted most expeditiously. Henceforth, benzene solutions were contained in closed systems, all exposed workers were given orange juice and vitamin-reinforced milk drinks twice a day; blood counts and urinary ascorbate excretions were taken weekly; and, most important of all, alcohol, acetone, and other solvents were used as alternatives to benzene wherever possible. This was probably one of the earliest uses of positive measures in industrial hygiene in the chemical industry, occurring as it did more than 55 years ago. The study of oxygen radicals and oxidative stress were very much in vogue at the time, and the role of ascorbic acid as an antioxidant was well recognized. Furthermore, the radiomimetic character of benzene toxicity indicated that, as with ionizing radiation, oxygen radicals or reactive oxygen species (ROS) were ultimately involved, and that vitamin C was probably a vital component of the biological defense against ROS and benzene toxicity.

Nearly 10 years later, after graduating in medicine and chemistry, and spending 4 years in the armed services, I made my second contact with benzene, this time as a research student with the late R.T. Williams at St. Mary's Hospital Medical School, London. Williams agreed that I should study all the known pathways of benzene metabolism, quantitatively and simultaneously, so as to obtain a balance-sheet of the metabolic fate of the chemical. Benzene was chosen, not only because of its known toxicity, but also because this was to be the parent compound and a model for more extensive programs of metabolism of a variety of aromatic chemicals. Furthermore, since benzene was known to be a radiomimetic toxin, it was considered that knowledge of its metabolism and mechanism of toxicity might reveal information concerning the mechanism(s) of radiation toxicity, a subject of great interest at that time, only 3 years after Hiroshima. Our research objectives included the following: *a*) to determine if benzene forms a mercapturic acid; *b*) to learn if benzene yields *cis-cis* and *cis-trans* isomers of muconic acid; *c*) to discover if benzene forms an epoxide or a dihydrodiol; *d*) to draw up a balance sheet for benzene metabolism; *e*) to synthesize ^{14}C-benzene and confirm the metabolism balance sheet; *f*) to determine if ^{14}C-benzene completely oxidized to $^{14}CO_2$; and *g*) to learn if its radiomimetic toxicity makes benzene a suitable model compound for studying biological radiation damage.

Initial Problems and Deficiencies

It is interesting today to reflect on how we financed this research program, since in the late 1940s almost no research grants were available on either side of the Atlantic. Consequently, there were almost no research assistants and all academic staff, including clinicians doing research, worked at the bench and fed and cleaned their own

This paper was presented at Benzene '95: An International Conference on the Toxicity, Carcinogenesis, and Epidemiology of Benzene held 17–20 June 1995 in Piscataway, New Jersey. Manuscript received 16 January 1996; manuscript accepted 14 June 1996.

Address correspondence to Dr. D. V. Parke, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, England. Telephone: 01483-300800. Fax: 01483-576978/300374.

Abbreviations used: GM, Geiger-Müller; GSH, glutathione; ROS, reactive oxygen species; UV, ultraviolet.

experimental animals. The work week was around 100 hr, with few free weekends and only 2 weeks vacation a year; little wonder that very few academics chose to undertake research. Money for animals and supplies came largely from commercial ventures, such as the marketing of ^{14}C-labeled chemicals synthesized by us but surplus to our requirements, marketing of 100% pure chemicals made "in house," e.g., cysteine, phenylglucuronide, and phenyl sulphate, and initially—while the cost was high—the recovery of penicillin from patients' urine and purifying this for reuse. Instruments, such as an ultraviolet (UV) spectrophotometer, a scintillation spectrometer for quantification of radioactive isotopes, and chromatography equipment, were not commercially available and had to be designed and fabricated in our own laboratory; in several instances this resulted in the establishment of new scientific instrument companies and a diversity of new products. Thus, these endeavors to meet research needs by pioneering scientific innovation resulted in successful commercial exploitation and development of several new industries.

A general problem that concerned us from the outset of this research was how to achieve and quantify chemical purity. The only methods then available were fractional recrystallization and distillation, and column chromatography on silica gel, alumina, or partially activated charcoal. Few purified solvents or chemical reagents were available commercially; indeed, as subsequent work showed, most commercially available chemicals were very crude mixtures. Benzene was purified by shaking with sulfuric acid to remove thiophene, then fractionally distilled and fractionally crystallized but was still far from pure as judged from melting-point studies and from later studies with ^{14}C-benzene when that was finally prepared. Other chemicals, especially the potential metabolites of benzene, namely, phenol, catechol, quinol (hydroquinone), and hydroxyquinol (1,2,4-trihydroxybenzene), were rigorously purified before use by preparation of derivatives, recrystallization to constant melting points, then hydrolyzed to regenerate the original compounds, which were further recrystallized to purity. In many cases the physical constants of the highly purified metabolites and their derivatives were significantly different from those previously recorded in the literature; this was particularly true where ^{14}C-labeled compounds had been synthesized and where constant specific radioactivity was the accepted criterion of purity. This gave rise to the view, often expressed by British and American chemists at that time, that the physical constants of aromatic compounds characterized before 1950 were probably incorrect and should be redetermined using specific radioactivity as the criterion of purity—an invitation that we firmly resisted so that we could concentrate our research efforts on the metabolism problems. Many years later, when we had adopted gas–liquid chromatography as the means of purification, with a symmetrical peak in two systems as a criterion of purity, we were amazed to receive a sample of ^{14}C-cyclohexane from Amersham (Amersham, U.K.) that contained not only ^{14}C-cyclohexene, ^{14}C-cyclohexadiene, and ^{14}C-benzene, but also ^{36}Cl-chlorobenzene as impurities. This discovery led to the realization that coelution of chemically similar compounds could prejudice the use of gas chromatography in purification procedures. Initial studies at Oxford with gas chromatography in the early 1960s showed that commercially recrystallized phenol, as used as a solvent for paper chromatography, was only some 60% pure, thus explaining our earlier need for rigorous purification of this benzene metabolite in isotope dilution studies. This great difficulty in obtaining pure compounds, i.e., 100% pure, greatly affected this research program, both in its inception and in the methodology we chose. Radioisotope labeling now was considered essential for the benzene metabolism study, and synthesis of ^{14}C-benzene was given high priority. Unlabeled benzene, for nonisotopic metabolism studies, was purified by fractional distillation, removal of thiophene, followed by fractional crystallization, and finally purification as the clathrate complex, as used in the purification of ^{14}C-benzene.

Synthesis of ^{14}C-Benzene

The only ^{14}C-labeled material available in the United Kingdom in 1948 was $Ba^{14}C_2$. We planned to use $Ba^{14}C_2$ to generate $^{14}C_2H_2$, then to pyrolyze this to $^{14}C_6H_6$ by passing through a red-hot tube, but the very poor yields led us to consider alternative synthetic approaches. Eventually, we concentrated our efforts on studying the metabolism of benzene using unlabeled material and left the radiosynthesis of ^{14}C-benzene to our chemistry collaborators.

^{14}C-Benzene with a single atom of ^{14}C per molecule was prepared eventually by ring closure of cyclopentane-^{14}C-carboxylic acid to ^{14}C-cyclohexane and dehydrogenation of the latter with Pt/C (*1*). ^{14}C-Benzene was quantified by end-window counting of the solid benzene–clathrate complex and checked by end-window counting of solid *m*-dinitrobenzene. The benzene–clathrate complex was formed by shaking benzene with a solution of nickel ammonium cyanide cooled in ice; the solid complex was filtered off, washed with water, ethanol, and diethyl ether, and dried in air (*2*). Dry distillation of the clathrate complex gave pure benzene.

Determination of the specific radioactivities of ^{14}C-metabolites was carried out by end-window counting, as liquid scintillation spectrometry had not been developed at that time. Indeed, researchers in the field decided to abandon scintillation counting because of the high background and lack of specificity. However, the potential advantages over end-window counting appeared so substantial that we decided to continue this line of research and were fortunate to find collaborators who, by the invention of coincidence circuitry and voltage discriminators, finally constructed a prototype Packard scintillation spectrometer. In subsequent metabolism studies with ^{14}C-labeled compounds, we were able to use the more specific, and more sensitive, scintillation spectrometry in place of end-window Geiger-Müller (GM) tube counting.

Benzene Eliminated Unchanged

Benzene was quantified by a colorimetric method based on trapping the exhaled benzene in a nitrating mixture, extracting the *m*-dinitrobenzene with methyl ethyl ketone, shaking with alkali to develop a purple color, and measurement of the light absorption in a Spekker absorptiometer using suitable light filters. The original method of Pearce et al. (*3*) was modified to optimize for sensitivity (*4*), which was 1 μg benzene with an accuracy of ± 5%.

Subsequently, when a variable wavelength UV spectrophotometer had been built, the benzene was trapped in ethanol, then quantified spectrophotometrically in the prototype Unicam SP500 (Cambridge Instruments, Cambridge, U.K.) by measurement of the absorption at 255.0 nm with a slit width of 0.4 mm and an ε_{max} of 240 (*5*). Also, when ^{14}C-benzene had been synthesized, a radiometric method was used; the benzene was trapped in ethanol, converted to the clathrate complex, then counted by the end-window GM tube method.

The experimental animals were chinchilla rabbits (23 kg bw); they were dosed

with benzene administered orally by gastric intubation or by ip injection. The animals were then placed in a Perspex chamber connected to the appropriate absorption train, and air was drawn through the system at 20 liters/hr for 20 to 30 hr. The absorption train was changed every 2 hr.

Agreement among the three methods of determination (colorimetric, spectrophotometric, and radiometric) of unchanged benzene was good (±10%). Elimination unchanged by rabbits was maximal during the first 12 hr after dosage and amounted to about 40% per dose at dose levels of 0.25 and 0.5 g/kg and 64% at 1.0 g/kg (*4*). The conclusions drawn from these studies were that the rabbit can metabolize benzene up to a limit of about 400 mg/kg/day and that with doses exceeding this, the excess benzene is exhaled in the expired air by a process similar to steam distillation (*4,6*). Negligible amounts of unchanged benzene (<0.01% dose) were found in rabbit urine.

Metabolism to L-Phenylmercapturic Acid

Phenylmercapturic acid, equivalent to 0.4% dose, had been isolated from the urine of rats dosed with benzene (*7*), but attempts to repeat this in other laboratories had failed. Furthermore, attempts to repeat the synthesis of L-phenylmercapturic acid (*8*) also failed. As a result, in the late 1940s the metabolism of benzene to phenylmercapturic acid was very much in doubt; indeed, even the role of mercapturic acids in the metabolism of other organic compounds was in question. One of the reasons for this was the failure of other laboratories to synthesize the phenylmercapturic acids as described by Zbarsky and Young (*8*). An investigation into the cause of this failure showed that the definitive paper had been erroneously altered by the journal editor, for throughout the paper, HCl had been changed to H_2SO_4 merely for the sake of uniformity, yet the chloride anion had been shown to be an essential catalyst for the synthesis. Moreover, our commercial cystine was grossly impure. As soon as sodium chloride was added to the reaction mixture and pure cystine was used, the various phenylmercapturic acids were synthesized in good yield; the validity of Zbarsky and Young's synthesis of the mercapturic acids and of their occurrence as metabolites of aromatic compounds were confirmed.

The excretion of phenylmercapturic acid in rabbit urine was quantified by the iodometric method of Stekol (*9*) and by a turbidimetric determination of the phenylmercapturic mercaptide (*10*). The two methods showed excellent agreement (± 2%) in the determination of standard amounts of synthetic L-phenylmercapturic acid added to rabbit urine, but the turbidimetric method was the more specific and was preferred over the iodometric titration, which gave high blank values with rabbit urine because of the excretion of dietary thiols. After dosing rabbits with benzene, the iodometric method gave 1.8 ± 0.2% dose as phenylmercapturic acid at 0.5 g benzene/kg bw and 1.2 ± 0.1% at 1.0 g/kg; the turbidimetric method gave 1.0 ± 0.1% and 0.8 ± 0.1% as phenylmercapturic acid at doses of benzene of 0.5 and 1.0 g/kg, respectively. Many attempts to isolate phenylmercapturic acid from the urine of rabbits dosed with benzene were unsuccessful, and the only crystalline material so obtained was benzoic acid.

Unequivocal proof of the formation of phenylmercapturic acid in the metabolism of benzene was obtained by oral administration of ^{14}C-benzene to rabbits. Reverse isotope dilution studies with synthetic L-phenylmercapturic acid gave material which, after repeated recrystallization to constant specific radioactivity, was equivalent to 0.73% of the dose of ^{14}C-benzene. Conversion of this material to phenylmercapturic mercaptide gave specific radioactivity equivalent to 0.70% dose and conversion to thiophenol *p*-nitrobenzoate gave activity equivalent to 0.74% dose. This figure of 0.7% dose from the radiobenzene study is in close agreement with the figures of 0.8 and 1.0% dose from the turbidimetric determination of phenylmercapturic acid formed from unlabeled benzene.

Metabolism to Muconic Acid

Muconic acid was first isolated as a metabolite of benzene by Jaffé (*11*) from the urine of dogs and rabbits. Although it was thought that if muconic acid were formed by opening of the benzene ring *in vivo* the *cis-cis* isomer should have been the initial product—as is obtained by the *in vitro* oxidative ring scission of catechol or phenol with peracetic acid—only the *trans-trans* isomer was isolated from the urine of animals dosed with benzene (*12*). The third geometric isomer of muconic acid, namely, the *cis-trans* isomer, was first characterized by Elvidge et al. (*13*) and was formed merely by recrystallization of the *cis-cis* isomer from water; in contrast, the *trans-trans* acid was formed only after UV irradiation of a solution of the *cis-cis* isomer in the presence of iodine as catalyst.

The objectives of the 1945 to 1950 study were therefore *a*) to develop a quantitative methodology for determination of each of the three isomers of muconic acid in urine; *b*) to isolate and characterize each isomer that was shown to be a metabolite of benzene; *c*) to determine the *in vitro* and *in vivo* stabilities of the three isomers to obtain an insight into the mechanism of formation of muconic acid from benzene; and *d*) to quantify the muconic acid isomers excreted after administration of ^{14}C-benzene to rabbits.

A colorimetric method, based on condensation of the muconic acid with phenol in the presence of H_2SO_4 to give a red pigment and soluble in ethanol, was developed to quantify each of the three isomers; *cis-cis* and *cis-trans* isomers give maximal red color when heated at 100°C for 6 hr; the *trans-trans* isomer, however, requires heating at 160°C for 20 min. Hence, the *cis* acids can be quantified when present in mixtures with the *trans-trans* acid; recoveries of all three isomers from urine were 100 ± 10% (*5,12*). *cis-cis*-Muconic acid added to rabbit urine was recovered quantitatively as a mixture of the *cis* acids; no *trans-trans* acid was formed. After ip injection of each of the three isomers of muconic acid into rabbits and quantification of the urinary excretion, the *trans-trans* acid was excreted unchanged equivalent to 52 ± 5% dose, the *cis-cis* acid was excreted as *cis* acids, equivalent to 66 ± 5% dose, and the *cis-trans* acid as *cis* acids equivalent to 55 ± 5% dose; no *trans-trans* acid was detected after injection of either of the *cis* acids. Hence, no evidence was found for isomerization of the *cis* acids to the *trans-trans* acid *in vivo*; furthermore, no evidence was found for selective loss of the *cis* acids added to rabbit urine *in vitro*. The quantitative method therefore indicates that *trans-trans*-muconic acid is a true metabolite of benzene in the rabbit, equivalent to 0.5% dose (0.15–1.0%) (*12*). Furthermore, dosing rabbits with phenol or catechol also resulted in the urinary excretion of *trans-trans*-muconic acid equivalent to 0.5 and 1.4% dose, respectively (*5*). The oxidative ring opening of benzene first gives rise to *cis-cis*-muconaldehyde, which then isomerizes to *cis-trans*- and *trans-trans*-muconaldehyde; the latter is oxidized *in vivo* to *trans-trans*-muconic acid, and this may be the actual route of formation of this paradoxical metabolite (*14*).

After oral dosing of benzene (7 g) to four rabbits, continuous ether extraction of the acidified urine gave 120 mg of crude crystalline material equivalent to 1.2%

dose, which was shown not to contain any *cis-cis-* or *cis-trans*-muconic acid. Recrystallization from ethanol gave the pure *trans-trans*-muconic acid equivalent to 0.1% dose of benzene, and this was further characterized as the benzhydryl ester. Finally, after administration of ^{14}C-benzene to rabbits, reverse isotope dilution studies showed that *trans-trans*-muconic acid was excreted in the urine equivalent to 1.3% of the dose.

cis-cis-Muconic acid had originally been prepared in the 1930s by the oxidation of phenol and catechol with dilute peracetic acid using 100 vol hydrogen peroxide. Twenty years later, because of the development of rocket fuel technology, 98% peroxide became available; therefore this was used in the synthesis of *cis-cis*-muconic acid from phenol to obtain increased yields. To decrease the risk of explosion, small amounts of reaction mixture (2 ml peracetic acid and 0.2 g phenol in 5 ml conical flasks) kept cool at 0°C were used with apparent safety. This was laborious and, to accelerate production, the individual volumes of peracetic acid were increased to 5 ml, with a total of 250 ml. However, temperature control was lost and a series of explosions ensued that wrecked the entire laboratory, so we reverted to the smaller individual volumes of reactant with complete safety.

Metabolism to Phenols

Phenol, catechol, and quinol (hydroquinone) had long been recognized as metabolites of benzene, but the oxidation of benzene to resorcinol, hydroxyquinol (1,2,4-trihydroxybenzene) and other trihydric phenols was uncertain. Attempts to devise specific colorimetric or spectrophotometric assays for the individual phenols all failed because of the interference of other phenols. Quantification of the individual phenolic metabolites of benzene was therefore dependent on the administration of ^{14}C-benzene, followed by reverse isotope dilution of the urines with a number of phenols and purification of crystalline derivatives. Phenol (23% dose) quinol (5%), catechol (3%), and hydroxyquinol (0.3%) were the major phenols present in the urines of rabbits dosed with ^{14}C-benzene; resorcinol, if present, was formed only in trace amounts (<0.3% dose), and phloroglucinol and pyrogallol were absent. The dihydrodiol of chlorobenzene had been isolated from rabbit urine at that time (*15*), so a similar isolation procedure was carried out on the urine of rabbits given ^{14}C-benzene. No dihydrodiol was ever detected. Nevertheless, it was presumed that the phenols had been formed by the dehydration of the corresponding diols, so that resorcinol and phloroglucinol were considered unlikely metabolites. Pyrogallol could have been a trace metabolite but was not detected, possibly because of polymerization of the quinone oxidation products, a process that may have led to low values for catechol, quinol, and hydroxyquinol despite the various precautions.

Determination of total conjugates in the urine of rabbits dosed with unlabeled benzene was the only approximation that could be made of the total phenols formed from unlabeled benzene. The total glucuronides were equivalent to 11% dose, and the ethereal sulfates were equivalent to 25% dose, a total of 36% (Table 1). This compares with a total of 31% being excreted as phenol, quinol, catechol, and hydroxyquinol in ^{14}C-benzene urine. This lower total might be due to the formation of *bis* conjugates of quinol, catechol, or hydroxyquinol.

Metabolism of ^{14}C-Benzene

As seen (Table 1), the quantitative balance sheet for the metabolism of unlabeled benzene is in good agreement with the more detailed picture obtained by dosing with ^{14}C-benzene (*16*). The totals in the expired air (47.5, 48.4%) and totals excreted in the urines (33, 38%) for the labeled and unlabeled benzenes are in excellent agreement.

Evidence was obtained that ^{14}C-benzene could be oxidized completely to $^{14}CO_2$ (1% dose), and consequently ^{14}C was incorporated into the body tissues to the extent of at least 5% dose. Indeed, it is most likely that the shortfall of some 15% dose in the overall balance sheet could be due to incorporation of the isotope ^{14}C into aliphatic carboxylic acids, formed as the result of oxidative ring scission, and the subsequent biosynthesis of these fatty acids into tissue components. In one rabbit experiment when ^{14}C-benzene was administered, the total residual radioactivity remaining in all of the tissues 48 hr after dosing was equivalent to some 16% dose (*5*).

Table 1. Metabolism of ^{14}C-benzene in rabbit.

Source	^{14}C-Benzene	Unlabeled benzene
Expired air		
Benzene	46.5	48.4
$^{14}CO_2$	1.0	–
Total in expired air	47.5%	48.4%
Urine		
Phenol	22.9[a]	
Catechol	2.9[a]	
Quinol	4.8[a]	
Resorcinol	< 0.3[a]	
Hydroxyquinol	0.3[a]	
L-Phenylmercapturic acid	0.4	1.0
trans-trans-Muconic acid	1.3	1.0
Total in urine	32.9%	38%
Feces	0.5%	–
Tissues	ca 5%	–
Total accounted for	86%	86%

[a]25 as ethereal sulfates; 11 as glucuronides. Data from Parke and Williams (*4,10,12,16*) and Parke (*5*).

Benzene and Oxygen Radicals

The known radiomimetic toxicity of benzene suggested, even some 50 years ago, that this solvent gives rise to the production of oxygen radicals ROS. Diethyl ether administered to rats also results in ROS production, glutathione depletion, and oxidative stress (*17,18*), for both diethyl ether and benzene activate the ROS-generating cytochrome P450, CYP2E1 (*19*) and can thus result in oxidative stress *in vivo*. However, benzene also generates ROS by the redox cycling of its quinone metabolites, which may explain the unique radiomimetic, hematopoietic toxicity of benzene, as CYP2E1 is not dominant in the bone marrow whereas myeloperoxidase is. Sources of ROS are as follows: *a*) ionizing radiation; *b*) iron and other redox metals; *c*) inflammation: activated leukocytes, infections, interleukins; *d*) eicosanoid biosynthesis (prostaglandin H synthase activity); *e*) redox cycling of quinones; *f*) futile cycling of cytochromes P450: P450-activated O_2 is partly inserted as ROS into the substrate and partly released as superoxide, etc.; and *g*) activation of CYP2E1: O_2 activation to ROS is preferred to the direct insertion of oxygen into the substrate. Bone-marrow phagocytes play a major role in benzene-induced hemotoxicity, and exposure of mice to benzene results in the activation of the phagocytes with increased ROS production, changed bone marrow progenitor-cell development, and increased production of interleukins (IL-1) and tumor necrosis factor (TNFα) (*20,21*).

Induction of CYP2E1 by benzene, ether, ethanol, acetone, and a variety of other small molecules, or by fasting, increases the metabolism of benzene and phenol to quinol and other myelotoxic metabolites (*22*) and also increases ROS production. ROS, from whatever origin, are the means of the metabolic activation of benzene and are the source of its toxicity, causing depletion of glutathione (GSH), oxidative stress, DNA damage, activation of protein kinase c, tissue necrosis, and

malignancy (*23*). The metabolic activation of benzene and the generation of ROS by CYP2E1 might be genetically dependent and polymorphic, as is the metabolism of ethanol by this cytochrome (*24*), so that benzene toxicity would show individual variation. Prostaglandin H synthase activity similarly metabolically activates benzene and phenol to toxic reactive intermediates, and consequently indomethacin inhibits benzene-induced bone-marrow toxicity (*25*). Myeloperoxidase in bone marrow also hydroxylates benzene to quinol and other reactive metabolites by generation of singlet oxygen (*26*).

The synergism of catechol and quinol in benzene-induced toxicity and leukemia (*27*) could involve a concerted action of redox cycling of quinol, generating ROS and thereby hydroxylating catechol to hydroxyquinol (benzene 1,2,4-triol). This would be followed by further redox cycling of hydroxyquinol, further ROS generation, interaction with GSH to form 2,5-dihydroxyphenylmercapturic acid (*28*), with consequent damage to hemopoietic tissues (*29*), and malignancy.

This generation of ROS from CYP2E1, redox cycling of the quinones, and other mechanisms have been shown in the case of ether anesthesia (*17,18*) to result in loss of GSH, oxidative stress, tissue inflammation and tissue necrosis (Figure 1), which may lead to chronic inflammatory disease such as rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, multiple system organ failure, and malignancy (*30,31*). Many chemicals, particularly substrates of CYP2E1 such as halothane and ether, initiate immune or inflammatory responses associated with ROS production and GSH depletion and are associated with many syndromes of chronic inflammation (*30*). Thus it is possible that benzene exposure may likewise be associated with various manifestations of systemic inflammation in addition to the known myelotoxicity and malignancy. Protection against ROS and oxidative stress is given by intracellular GSH and by dietary ascorbic acid, tocopherol, and other radical-trapping antioxidants (*30*).

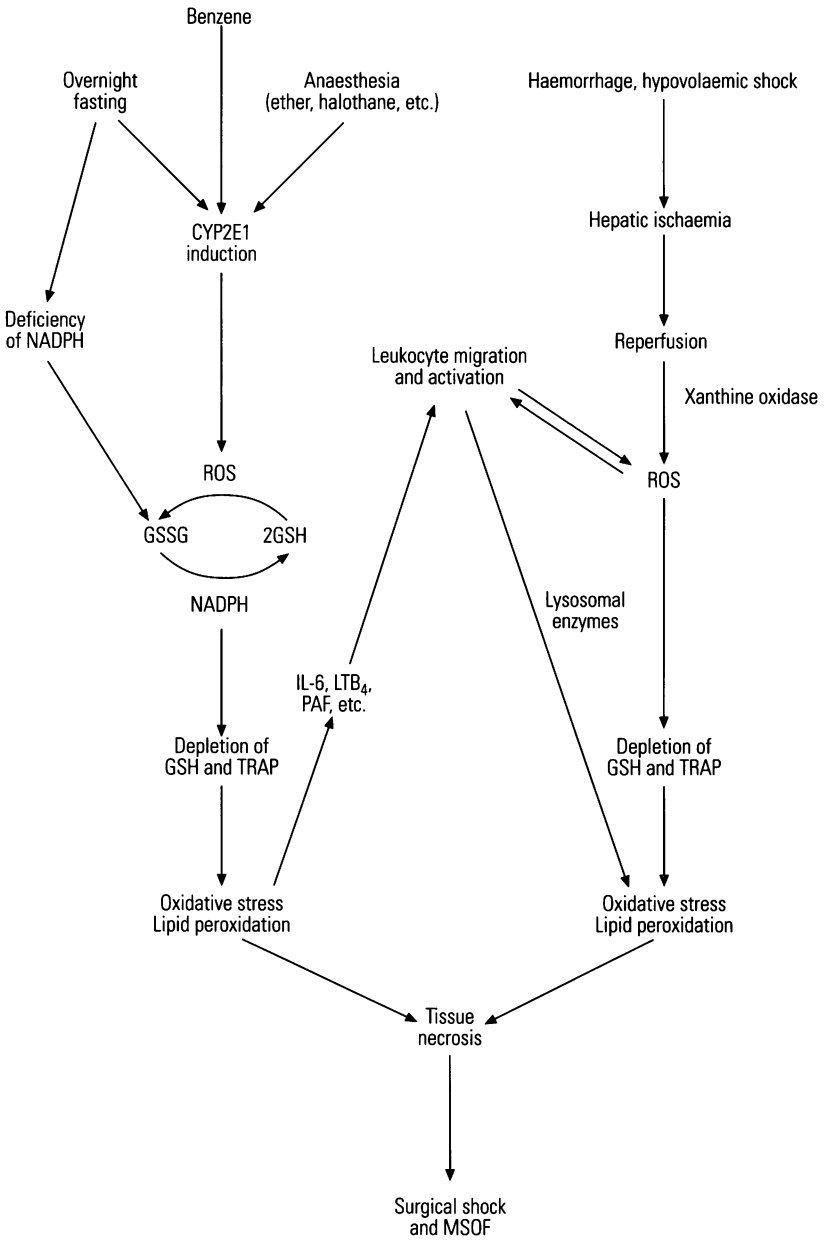

Figure 1. Mechanisms of oxidative stress and tissue inflammation. ROS are generated from cytochrome P4502E1 (CYP2E1), stimulated by exposure to benzene, ether, and other small molecules, and by fasting. The ROS oxidize intracellular GSH to GSSG, which is lost from the cell unless GSH is regenerated by glutathione reductase plus NADPH. Loss of GSH and other antioxidants (TRAP = total radical antioxidant parameter = tocopherols, ascorbic acid, retinoids, etc.) results in oxidative stress, lipid peroxidation, release of interleukins (IL-6), leukotrienes (LTB_4), platelet-activating factor (PAF), etc. This, in turn, leads to leukocyte activation and migration into tissues, with generation of further ROS, depletion of GSH and TRAP, resulting in surgical shock and multiple system organ failure (MSOF). Hence, exposure to benzene might result in conditions associated with chronic systemic inflammation.

Conclusions

It has long been known that the unique radiomimetic myelotoxicity of benzene is associated with its metabolism and that the difference in toxicity between benzene and the halobenzenes is associated with fundamental differences in their metabolic fates (*32*). Whereas the hepatotoxic chlorobenzene and bromobenzene are oxidized by various cytochromes P450 to form a relatively stable 3,4-epoxide that yields the corresponding dihydrodiol, catechol, and mercapturic acid as the major metabolites (*15*), the myelotoxic, leukemogenic benzene is oxidized by ROS from CYP2E1 to yield a metastable radical that rearranges to form phenol as the major metabolite, with only traces of catechol (3%) and phenylmercapturic acid (1%). Deactivation of the aromatic ring by the halogen substituent thus facilitates the insertion of an activated oxygen from a cytochrome P450, and this allows epoxidation and

consequent metabolic detoxication. By contrast, benzene appears to be metabolized with difficulty; and as with ethanol, diethyl ether, carbon tetrachloride, acetone, and many other small molecules that are resistant to oxidative metabolism by the cytochromes P450, it is metabolized by CYP2E1 by generating ROS. The ROS also mediate malignancy and form quinones that undergo redox cycling to generate ROS in bone marrow, mediating myelotoxicity (*19,25*). The identification of other CYP2E1 substrates that are likely to manifest myelotoxicity and malignancy is now well advanced and depends on determination of the molecular diameter of the chemical and of the energies of the molecular orbitals (*23,33*). Thus, our knowledge of benzene metabolism and toxicity has continued to evolve over more than 50 years and is now enabling the prediction of chemicals with similar toxicity.

REFERENCES

1. Turner HS. A convenient sythesis of carbon-labelled benzene. Nature 168:73–74 (1951).
2. Evans RF, Ormrod I, Goalby BB, Staveley LAK. Purification of benzene. J Chem Soc 3346 (1950).
3. Pearce SJ, Schrenk HH, Yant WP. Microcolorimetric determination of benzene in blood and urine. Rep Investig No 3302 Chem Abstr 30:5600 (1936).
4. Parke DV, Williams RT. Studies in detoxication. 30: The metabolism of benzene. a) The determination of benzene. b) The elimination of benzene unchanged by rabbits. Biochem J 46:236–243 (1950).
5. Parke DV. The Metabolism of Aromatic Compounds. PhD Thesis, London:University of London, 1952.
6. Azouz WM, Parke DV, Williams RT. Studies in detoxication. 42: Fluorobenzene. Spectrophotometric determination of the elimination of unchanged halobenzene by rabbits. A comparison of the oxidation *in vivo* of fluorobenzene and of benzene. Biochem J 50:702–706 (1952).
7. Zbarsky SH, Young L. Mercapturic acids. III: The conversion of benzene to phenylmercapturic acid in the rat. J Biol Chem 151:487–492 (1943).
8. Zbarsky SH, Young L. Mercapturic acids. I: Synthesis of phenyl-*l*-cysteine and L-phenylmercapturic acid. J Biol Chem 151:211–215 (1943).
9. Stekol JA. The determination of *p*-bromophenylmercapturic acid in the urine of dog. J Biol Chem 113:279–288 (1936).
10. Parke DV, Williams RT. Studies in detoxication. 38: The metabolism of benzene. a) The determination of phenylmercapturic acid in urine. b) The mercapturic acid excretion by rabbits receiving benzene. Biochem J 48:624–628 (1951).
11. Jaffé M. Cleavage of the benzene ring in the organism. 1. The excretion of muconic acid in the urine after ingestion of benzene. Z Physiol Chem 62:58–67 (1909).
12. Parke DV, Williams RT. Studies in detoxication 44. The metabolism of benzene. The muconic acid excreted by rabbits receiving benzene. Determination of the isomeric muconic acids. Biochem J 51:339–348 (1952).
13. Elvidge JA, Linstead RP, Sims P, Orkin BA. Third isomeric (*cis-trans*)muconic acid. J Chem Soc 2235–2241 (1950).
14. Latriano L, Goldstein BD, Witz G. Formation of muconaldehyde, an open-ring metabolite of benzene in mouse liver microsomes: an additional pathway for toxic metabolites. Proc Natl Acad Sci USA 83:8356–8360 (1986).
15. Smith JN, Spencer B, Williams RT. Detoxication. XXXIV. Metabolism of chlorobenzene in the rabbit. Isolation of dihydrodihydroxychlorobenzene, *p*-chlorophenylglucuronide, 4-chlorocatechol glucuronide and *p*-chlorophenylmercapturic acid. Biochem J 47:284–293 (1950).
16. Parke DV, Williams, RT. The metabolism of benzene containing ^{14}C-benzene. Biochem J 54:231–238 (1953).
17. Liu PT, Ioannides C, Symons AM, Parke DV. Role of tissue glutathione in the prevention of surgical trauma. Xenobiotica 23:899–911 (1993).
18. Liu PT, Ioannides C, Shavila J, Symons AM, Parke DV. Effects of ether anaesthesia and fasting on various cytochromes P450 of rat liver and kidney. Biochem Pharmacol 45:871–877 (1993).
19. Johansson I, Ingelman-Sundberg M. Hydroxyl radical-mediated cytochrome P450-dependent metabolic activation of benzene in microsomes and reconstituted enzyme systems from rabbit liver. J Biol Chem 258:7311–7316 (1983).
20. MacEachern L, Laskin DL. Bone marrow phagocytes, inflammatory mediators and benzene toxicity. In: Xenobiotics and Inflammation (Schook LB, Laskin DL, eds). San Diego:Academic Press, 1994;149–171.
21. MacEachern L, Snyder R, Laskin DL. Alterations in the morphology and functional activity of bone-marrow phagocytes following benzene treatment of mice. Toxicol Appl Pharmacol 117:147–154 (1992).
22. Schrenk D, Ingelman-Sundberg M, Bock KW. Influence of P4502E1 induction on benzene metabolism in rat hepatocytes and on biliary excretion. Drug Metab Dispos Biol Fate Chem 20:137–141 (1992).
23. Parke DV. The cytochromes P450 and mechanisms of chemical carcinogenesis. Environ Health Perspect 102:852–853 (1994).
24. Tsutsumi M, Wang J-S, Takase S, Takada A. Hepatic messenger RNA contents of cytochrome P4502E1 in patients with different P4502E1 genotypes. Alcohol Alcohol 29(Suppl 1):29–32 (1994).
25. Kalf GH, Schlosser MJ, Renz JF, Pirozzi SJ. Prevention of benzene-induced myelotoxicity by nonsteroidal antiinflammatory drugs. Environ Health Perspect 82:57–64 (1989).
26. Subrahmanyam V, Kolachana P, Smith MT. Hydroxylation of phenol to hydroquinone catalysed by a human myeloperoxidase-superoxide complex: possible implications in benzene-induced myelotoxicity. Free Radic Res Commun 15:285–296 (1991).
27. Robertson ML, Eastmond DA, Smith MT. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. Mutat Res 249:201–209 (1991).
28. Nerland DE, Pierce M Jr. Identification of *N*-acetyl-*S*-(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol and hydroquinone. Drug Metab Dispos Biol Fate Chem 18:958–961(1990)
29. Zhang L, Robertson ML, Kalachana P, Davis AJ, Smith MT. Benzene metabolite, 1,2,4-benzenetriol, induces micronuclei and oxidative damage in human lymphocytes and HL60 cells. Environ Mol Mutagen 21:339–348 (1993).
30. Parke AL, Parke DV. The pathogenesis of inflammatory disease: surgical shock and multiple system organ failure. Inflammopharmacology 3:1079–1099 (1995).
31. Parke AL, Ioannides C, Lewis DFV, Parke DV. Molecular pathology of drug-disease interactions in chronic autoimmune inflammatory diseases. Inflammopharmacology 1:1–36 (1991).
32. Parke DV, ed. The Biochemistry of Foreign Compounds. Oxford:Pergamon Press, 1968;216–217.
33. Lewis DFV, Ioannides C, Parke DV. A retrospective evaluation of COMPACT predictions of the outcome of NTP rodent carcinogenicity testing. Environ Health Perspect 103:178–184 (1995).